

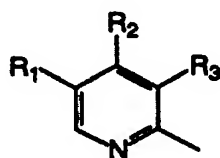
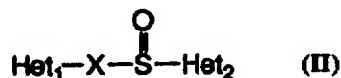


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

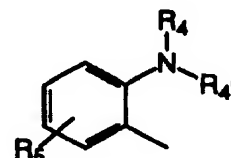
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<b>(21) International Application Number:</b> PCT/SE95/01415 <b>(22) International Filing Date:</b> 27 November 1995 (27.11.95) <b>(30) Priority Data:</b> 9423970.4 28 November 1994 (28.11.94) GB <b>(71) Applicant (for all designated States except US):</b> ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> HOLT, Robert [GB/GB]; 3 Village Way, Kirkby, Fleetham, Northallerton DL7 0TW (GB). LINDBERG, Per [SE/SE]; Gundas gata 40, S-431 51 Mölndal (SE). REEVE, Christopher [GB/GB]; 28 Roseberry Crescent, Great Ayton, Middlesborough, Cleveland TS9 6ER (GB). TAYLOR, Stephen [GB/GB]; Hillview House, Cleasby, Darlington, CO. Durham DL2 2QY (GB). <b>(74) Agent:</b> ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).		<b>(81) Designated States:</b> AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).  <b>Published</b> <i>With international search report.</i>

**(54) Title:** ENANTIOSELECTIVE PREPARATION OF PHARMACEUTICALLY ACTIVE SULFOXIDES BY BIOOXIDATION**(57) Abstract**

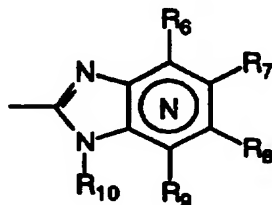
A compound of formula (II) is prepared either as a single enantiomer or in an enantiomerically enriched form, wherein Het<sub>1</sub> is (a) or (b) and Het<sub>2</sub> is (c) or (d) and X is (e) or (f) wherein N in the benzimidazole moiety means that one of the carbon atoms substituted by R<sub>6</sub>-R<sub>9</sub> optionally may be exchanged for an unsubstituted nitrogen atom; R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy; R<sub>4</sub> and R<sub>4</sub>' are the same or different and selected from hydrogen, alkyl, aralkyl; R<sub>5</sub> is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy; R<sub>6</sub>-R<sub>9</sub> are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxy carbonyl, oxazolyl, trifluoroalkyl or adjacent groups R<sub>6</sub>-R<sub>9</sub> may complete together with the carbon atoms to which they are attached optionally substituted ring structures; R<sub>10</sub> is hydrogen or alkoxy-carbonyloxymethyl; R<sub>11</sub> is hydrogen or forms an alkylene chain together with R<sub>3</sub>; R<sub>12</sub> and R<sub>13</sub> are the same or different and selected from hydrogen, halogen or alkyl, by a method comprising stereoselective biooxidation of the pro-chiral sulfide counterpart compound.



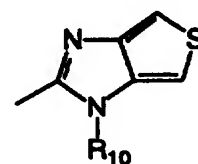
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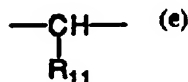
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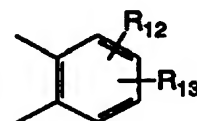
(c)



(d)



(e)



(f)

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Enantioselective preparation of pharmaceutically active sulfoxides by biooxidation

- 5 The present invention relates to a method of preparing compounds as defined below, either as a single enantiomer or in an enantiomerically enriched form, by biooxidation of their sulphide equivalents.

### Background to the Invention

- 10 The racemic form of the compounds prepared by the method of the present invention are known compounds. Some of the compounds are also known in single enantiomeric form. The compounds are active H<sup>+</sup>K<sup>+</sup>ATPase inhibitors and they, including their pharmaceutically acceptable salts, are effective acid secretion inhibitors, and known for use as antiulcer agents. The compounds, which include
- 15 the known compounds omeprazole (compound of formula (IIa) below), lansoprazole (compound of formula (IIc) below) and pantoprazole (compound of formula (IIb) below), are known for example from European Patent Specifications EP 5129 and 124495, EP 174726 and EP 166287.
- 20 These compounds, being sulfoxides, have an asymmetric centre in the sulfur atom, i.e. exist as two optical isomers (enantiomers). It is desirable to obtain compounds with improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile such as a lower degree of interindividual variation.
- 25 The separation of enantiomers of omeprazole in analytical scale is described in e.g. J. Chromatography, 532 (1990), 305-19. Also the separation of enantiomers of compounds, including omeprazole and pantoprazole, is described in German Patent Specification DE 4035455.

Recently there has been a great deal of literature published relating to the synthesis of optically active compounds using biocatalysts. The majority of this work has been aimed at finding routes to single enantiomer forms of pharmaceuticals. The reactions receiving most attention have been those involved in the preparation of esters, acids and alcohols due to the general utility of these functionalities in synthesis and also because the biocatalysts are readily available.

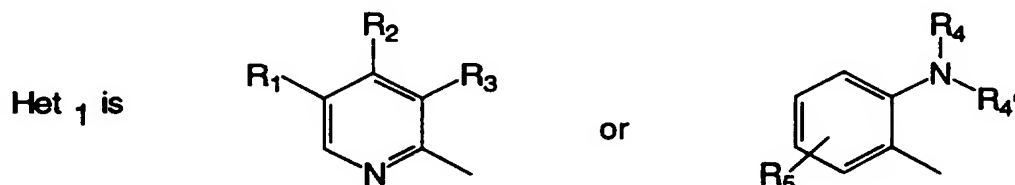
Studies on the synthesis of optically active sulfoxides are relatively rare partly due to the small number of pharmaceuticals containing sulfoxide groups and partly due to the fact that enzymes that react with the sulphur centre are not available commercially. The synthesis of optically active sulfoxides has been described in Holland, H.L. (1988) Chem. Rev. 88, 473-483 and Phillips, R.S. and Sheldon W.M., Enzyme Microb. Technol., 1981, Vol. 3, January, 9-18.

### 15 Description of the Invention

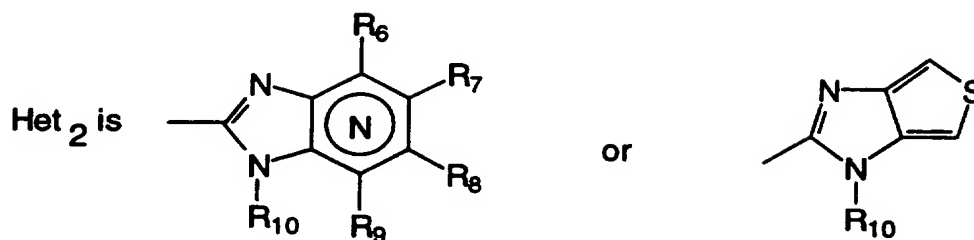
According to the present invention there is provided a method of preparing a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:



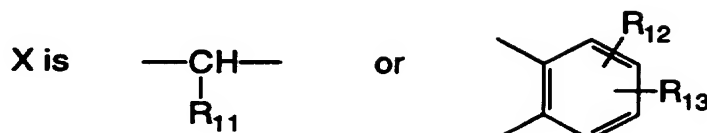
wherein



25 and



and



wherein:

5

N in the benzimidazole moiety means that one of the carbon atoms substituted by R<sub>6</sub>-R<sub>9</sub> optionally may be exchanged for an unsubstituted nitrogen atom;

10 R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

R<sub>4</sub> and R<sub>5</sub> are the same or different and selected from hydrogen, alkyl, aralkyl;

15 R<sub>6</sub> is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

20 R<sub>6</sub> - R<sub>9</sub> are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R<sub>6</sub> - R<sub>9</sub> may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

R<sub>10</sub> is hydrogen or alkoxycarbonyloxymethyl;

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$R_{11}$  is hydrogen or forms an alkylene chain together with  $R_3$ ;

$R_{12}$  and  $R_{13}$  are the same or different and selected from hydrogen, halogen or alkyl, which method comprises stereoselective biooxidation of the pro-chiral sulfide

5 counterpart compound.

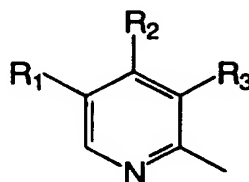
The compounds of formula (II) are active  $H^+K^+$ ATPase inhibitors. By the method of the invention these compounds, which are sulfoxides, are obtained in single enantiomer form or such that one enantiomeric form is present in excess leading to an optically active product, by stereoselective biooxidation of the pro-chiral starting sulfide counterpart compound.

In the above definitions alkyl groups or moieties may be branched or straight chained or comprise cyclic alkyl groups, for example cycloalkylalkyl.

15

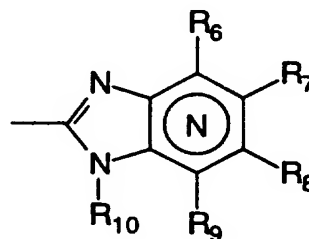
Preferably:

Het<sub>1</sub> is



and

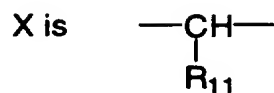
Het<sub>2</sub> is



20

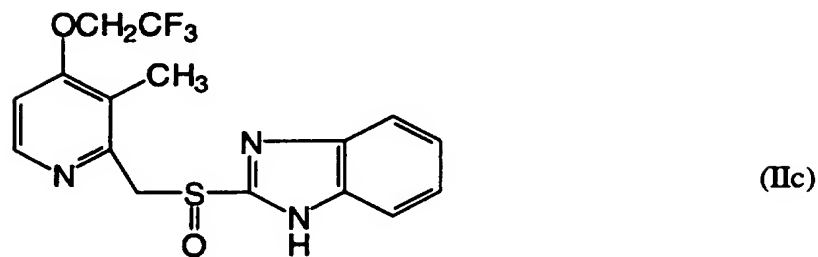
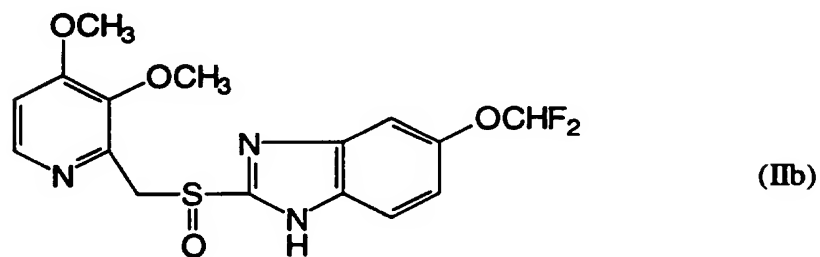
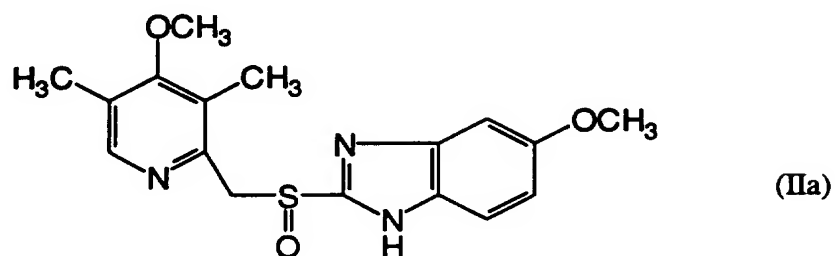
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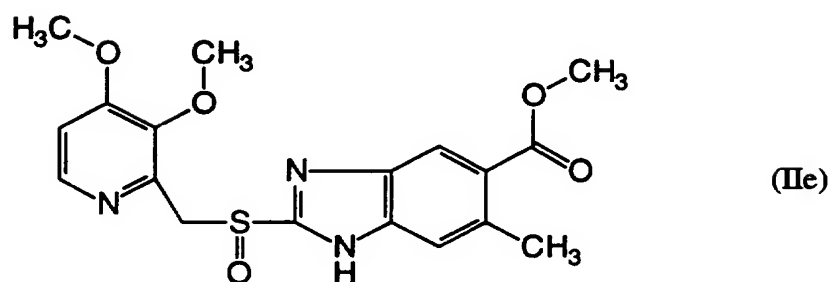
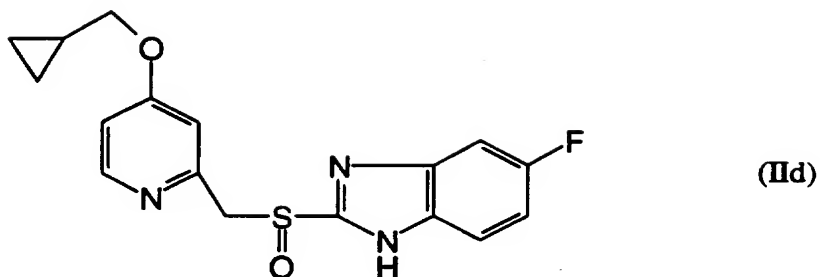
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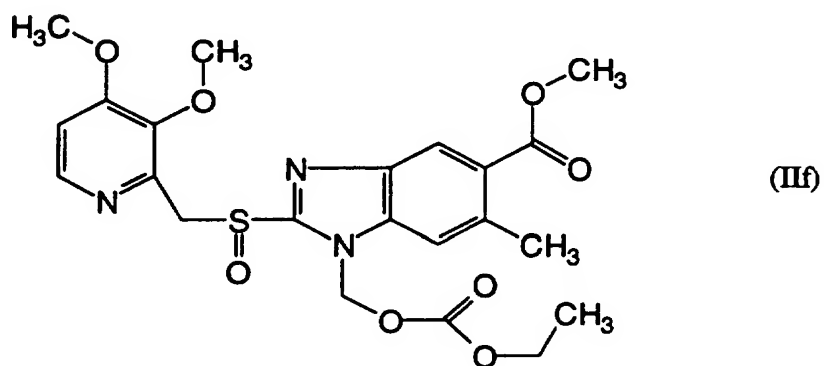
(wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  to  $R_9$ ,  $R_{10}$  and  $R_{11}$  are as defined above).

Most preferably the compounds of formula (II) are compounds of the formula (IIa)  
5 to (IIe):





- 5 An example of a compound of formula (II) wherein  $R_{10}$  is alkoxy-carbonyloxymethyl is



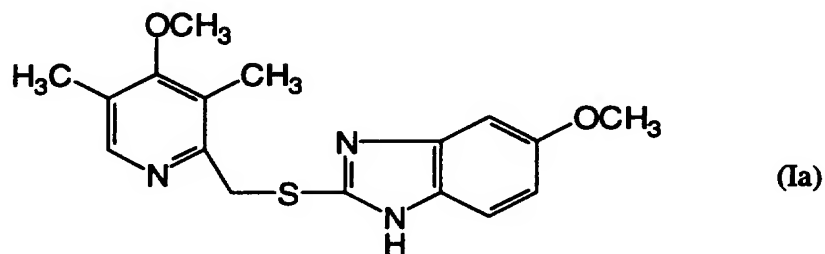
- 10 The starting pro-chiral sulfides used in the method of the present invention are of the formula:



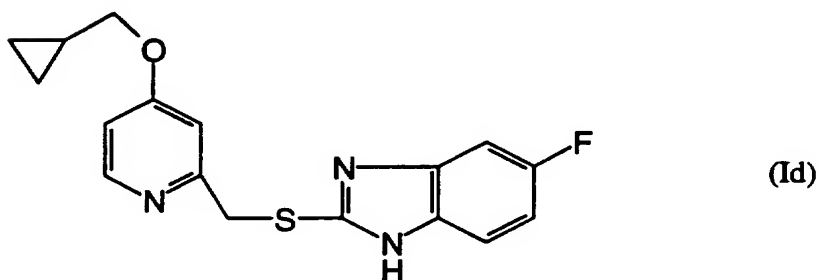
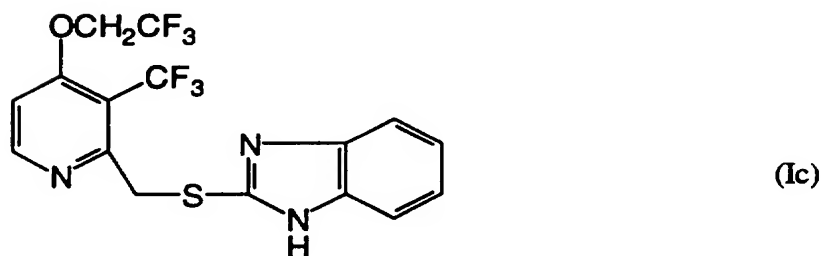
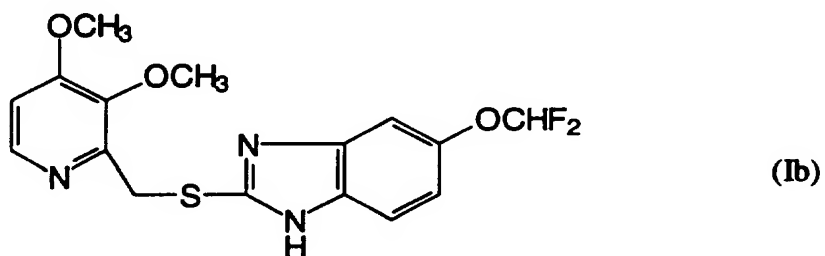
wherein  $\text{Het}_1$ , X and  $\text{Het}_2$  are as defined above.



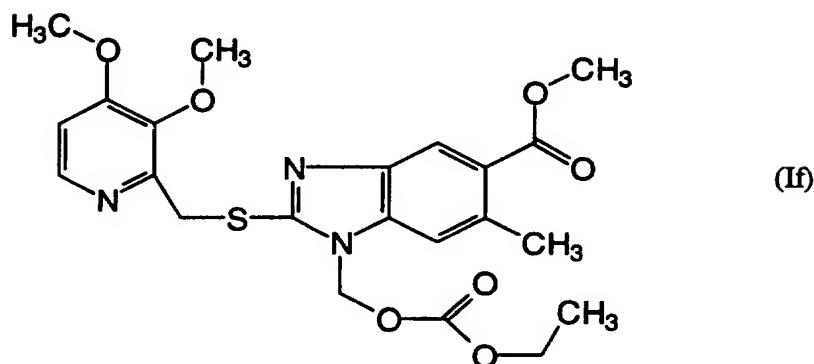
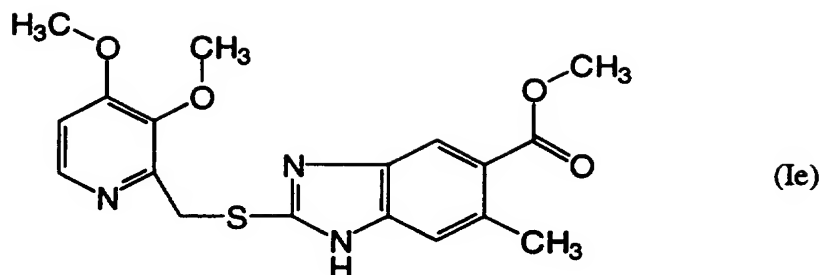
In order to obtain each of the above compounds (IIa)-(IIf), the following starting compounds of formula (Ia) to (If), respectively will be required:



5



10



- 5 The compounds prepared by the method of the invention possess a stereogenic (asymmetric) centre which is the sulfur atom which forms the sulfoxide group between the Het<sub>1</sub>-X-moiety and the Het<sub>2</sub>-moiety.

10 The stereoselective biooxidation according to the present invention may be carried out using a microorganism or an enzyme system derivable therefrom. Suitable microorganisms may be selected from alkane oxidisers including Arthrobacter petroleophagus, Brevibacterium paraffinolyticum, and Acinetobacter species, alkene oxidisers such as Mycobacterium species, and a variety of fungal species particularly Penicillium species (Penicillium frequentans).

15

According to one embodiment of the invention the method comprises contacting the pro-chiral sulfide counterpart compound with a microorganism which is

Penicillium frequentans

Rhizopus stolonifer

Cunninghamella elegans

Ustilago maydis

5 Arthrobacter petroleophagus

Brevibacterium paraffinolyticum

Acinetobacter sp.

Mycobacterium sp.

or Aspergillus niger

10 Preferably the microorganism is:

Penicillium frequentans BPFC 386, 585, 623, 733

Rhizopus stolonifer BPFC 1581

Ustilago maydis BPFC 1198, 6333

Arthrobacter petroleophagus ATCC 21494

15 Brevibacterium paraffinolyticum ATCC 21195

Actinetobacter sp. NCIMB 9871

Mycobacterium sp. BPCC 1174, 1178, 1179, 1186, 1187

or Aspergillus niger BPFC 32

20 The microorganisms may be grown on suitable medium containing an appropriate carbon source such as octane, ethene, cyclohexanone or glucose for example.

The compounds of formula (II) are generally acid labile and thus the use of acid conditions is to be avoided. Generally the method according to the invention may  
25 be carried out at a pH of 7.6 to 8, suitably about 7.6, and at temperature of 25-35°C, suitably about 28°C.

The present invention will now be illustrated with reference to the Examples.

**EXAMPLE 1**

The following microorganisms were screened for sulfoxidation activity against compounds of formula (Ia):

5

Penicillium frequentans BPFC 386

Penicillium frequentans BPFC 585

Penicillium frequentans BPFC 623

Penicillium frequentans BPFC 733

10 

Rhizopus stolonifer BPFC 1581

Ustilago maydis BPFC 1198

Ustilago maydis BPFC 6333

Arthrobacter petroleophagus ATCC 21494

Brevibacterium paraffinolyticum ATCC 21195

15 

Acinetobacter sp NCIMB 9871

Mycobacterium sp BPCC 1174

Mycobacterium sp BPCC 1178

Mycobacterium sp BPCC 1179

Mycobacterium sp BPCC 1186

20 

Mycobacterium sp BPCC 1187

**Growth Conditions**25 

The growth conditions for the above microorganisms were as follows. The following fungi:

Penicillium frequentans BPFC 386

Penicillium frequentans BPFC 585

Penicillium frequentans BPFC 623

30 

Penicillium frequentans BPFC 733

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Rhizopus stolonifer BPFC 1581

Ustilago maydis BPFC 1198

Ustilago maydis BPFC 6333

- 5 were grown in 200 ml of sterile liquid medium (I) with the composition of (per litre)  $K_2HPO_4$  (1.9g),  $NaH_2PO_4 \cdot 2H_2O$  (2.02g), ammonium sulfate (1.8g), magnesium sulfate (0.2g), ferric chloride (0.97 mg), and trace elements solution (1 ml) pH 7.2. The composition of the trace elements solution used was as follows (in g/l):

10	$CuSO_4 \cdot 5H_2O$	0.02
	$MnSO_4 \cdot 4H_2O$	0.1
	$ZnSO_4 \cdot 7H_2O$	0.1
	$CaCO_3$	1.8

- 15 The above medium was supplemented with 0.2% w/v yeast extract and 2.2% w/v glucose. The medium contained in 1L baffled flasks was inoculated either by adding a suspension of spores in sterile distilled water or by the addition of a plug of agar containing the fungi from a Sabouraud Dextrose plate. Fungi were grown at 28°C on a rotary shaker at 150 rpm for 48 hours. With the exception of Ustilago maydis,  
20 the fungal biomass obtained from liquid culture was harvested by filtration on a Whatman Grade 113 filter paper and washed on the filter with 50 mM sodium phosphate buffer, pH7.6. Ustilago maydis was harvested by centrifuging for 20 minutes at 8,000 rpm and 4°C. The biomass was washed by resuspending in 50 mM sodium phosphate buffer, pH 7.6 and centrifuging as above.

25

The bacteria were grown with the sources of carbon shown in Table 1:

**TABLE 1**

Microorganism	Carbon Source
<i>Arthrobacter petroleophagus</i> ATCC 21494	Octane
<i>Brevibacterium paraffinolyticum</i> ATCC 21195	Octane
<i>Acinetobacter</i> sp NCIMB 9871	Cyclohexanone
<i>Mycobacterium</i> sp BPCC 1174, 1178, 1179, 1186, 1187	Ethene

5 The growth of *Acinetobacter* sp. NCIMB 9871 on cyclohexanone was performed in 100 ml of liquid medium (I) in a 500 ml baffled flask containing a centre well. Cyclohexanone was placed in the centre well. The microorganism was grown at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

10 Growth of *Arthrobacter petroleophagus* ATCC 21494 and *Brevibacterium paraffinolyticum* ATCC 21195 on octane was performed in 200 ml of liquid medium (I) containing 0.2% w/v yeast extract in a 1 L baffled flask. Octane (1ml) was added directly to the medium without sterilization. The above microorganisms were grown at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

15 *Mycobacterium* sp BPCC 1174, 1178, 1179, 1186 and 1187 were grown in 500 ml liquid medium (I) in a 2L non-baffled flask fitted with a rubber bung. The flask was partially evacuated and then charged with ethene. Growth was conducted at 28°C on a rotary shaker at 150 rpm for 7 days.

20 Growth of *Arthrobacter petroleophagus* ATCC 21494 and *Brevibacterium paraffinolyticum* ATCC 21195 was also performed on glucose. Each microorganism was inoculated into 200 ml medium (I) containing 0.2% w/v yeast extract and 2.2% w/v glucose. Growth was performed at 28°C on a rotary shaker at 150 rpm for 24-48 hours.

25

All bacteria were harvested from liquid medium by centrifuging at 8,000 rpm and 4°C for 20 minutes. Cells were washed by resuspending in 50 mM sodium phosphate buffer, pH 7.6 followed by centrifuging as above.

## 5 Biooxidation Reactions

Biotransformations were performed for each microorganism in 50mM sodium phosphate buffer, pH 7.6 with 5-10 g/l dry cell weight and a substrate concentration of 1 g/l. The cells were incubated with the compound of formula (Ia) on a rotary  
10 shaker at 28°C for 18-20 hours.

Samples were removed from the biotransformation and either centrifuged or filtered to remove biomass and analysed directly.

## 15 Detection of Products

The biooxidation of the compound of formula (Ia) was followed by reverse phase HPLC on a Spherisorb S5-ODS2 reverse phase column eluted with a 50:50 mixture of acetonitrile and 25mM sodium phosphate buffer, pH 7.6 at a flow rate of 0.8  
20 ml/min. Under such conditions the compounds of formulae (IIa) and (Ia) were well resolved with retention times of 5.2 and 9.8 minutes respectively. Both compounds were detected at a wavelength of 300 nm.

The enantiomeric composition of the compound of formula (IIa) formed was  
25 investigated by the following method. After removal of biomass the aqueous media was extracted with two volumes of ammonia saturated dichloromethane. The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford a pale brown solid. Then the enantiomeric composition of sulfoxide was determined by chiral HPLC on a  
30 Chiralpak AD Column under the following conditions:

Column Chiralpack AD 250 mm x 4.6 mm interior

diameter with 50 mm guard column

- 5 Eluent Hexane:Ethanol:Methanol (40:55:5% V/V)  
 Flow 1.0 ml/min  
 Injection Volume 20 $\mu$ l  
 Wavelength 300 nm  
 Retention times
- 10 Compound of formula (Ia) 5.1 min  
 Compound of formula (IIa):  
 (+) Enantiomer 8.5 min  
 (-) Enantiomer 13.4 min
- 15 The following results were obtained:

**TABLE 2**

Microorganism	Compound of Formula (IIa) (ppm)	Enantiomeric excess (%)	Enantiomer ((+) or (-))
<i>Penicillium frequentans</i> BPFC 386	23	>99	(-)
<i>Penicillium frequentans</i> BPFC 585	2.1	>99	(-)
<i>Penicillium frequentans</i> BPFC 623	3.0	95	(-)
<i>Penicillium frequentans</i> BPFC 733	2.6	87	(-)
<i>Rhizopus stolonifer</i> BPFC 1581	3.0	56	(-)
<i>Ustilago maydis</i> BPFC 1198	8.0	88	(-)
<i>Ustilago maydis</i> BPFC 6333	34.0	99	(-)
<i>Arthrobacter petroleophagus</i> ATCC 21494	24.0	96	(-)
<i>Brevibacterium paraffinolyticum</i> ATCC 21195	13.0	>99	(-)
<i>Acinetobacter</i> sp NCIMB 9871	0.4	17	(-)
<i>Mycobacterium</i> sp BPCC 1174	10.0	97	(-)
<i>Mycobacterium</i> sp BPCC 1178	3.3	93	(-)
<i>Mycobacterium</i> sp BPCC 1179	9.0	96	(-)
<i>Mycobacterium</i> sp BPCC 1186	11.0	97	(-)
<i>Mycobacterium</i> sp BPCC 1187	6.0	96	(-)

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The enantiomeric excess value gives an indication of the relative amounts of each enantiomer obtained. The value is the difference between the relative percentages for the two enantiomers. Thus, for example, when the percentage of the (-) enantiomer of the formed sulfoxide is 97.5% and the percentage for the (+) enantiomer is 2.5%, the enantiomeric excess for the (-) enantiomer is 95%.

With Arthrobacter petroleophagus ATCC 21494 and Brevibacterium paraffinolyticum ATCC 21195 the stereoselectivity of the biooxidation was unaffected by the choice of carbon source used for growth (octane and glucose).

## EXAMPLE 2

Compounds of formula (Id) and (Ie) were screened against a range of microorganisms for the production of the corresponding sulfoxides. The growth of microorganisms and subsequent biotransformations were performed as in Example 1 except that the reaction times were as listed in Tables 5 and 6. Aspergillus niger BPFC 32 was grown in the same way as the fungi were grown in Example 1.

## Detection of Products

The biooxidation of the compounds of formula (Id) and (Ie) was followed by reverse phase HPLC as in Example 1 except that the retention times were as follows:

**TABLE 3**

Compound of formula	Retention time (min)
Id	13.7
IId	5.0
Ie	9.4
Ile	4.3

The enantiomeric composition of the compounds of formula (IId) and (Ile) was investigated by the method of Example 1 except in the chiral HPLC the solvent compositions, flow rates and retention times were as follows:

**TABLE 4**

Compound of formula	Solvent Composition	Flow rate (ml/min)	Retention Time
IId	Hexane/Ethanol (70:30% v/v)	1.0	12.9 (Enantiomer A) 21.7 (Enantiomer B)
	Hexane/Ethanol/Methanol (40:55:5% v/v)	1.0	7.4 (Enantiomer A) 10.6 (Enantiomer B)
Ile	Hexane/Ethanol (70:30% v/v)	1.0	26.0 (Enantiomer A) 30.5 (Enantiomer B)

In Table 4 the first enantiomer eluted is referred to as enantiomer A and second as enantiomer B. The results are summarised in Tables 5 and 6.

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**TABLE 5**

Microorganism	Reaction time (h)	Aqueous concentration (PPM)		E.e. %	Enantiomer
		Compound of formula (Id)	Compound of formula (IId)		
<i>Mycobacterium sp.</i> BPCC 1174	42	5	16.7	>99	A
<i>Mycobacterium sp.</i> BPCC 1178	42	5.9	14.4	>99	A
<i>Mycobacterium sp.</i> BPCC 1179	42	6.6	17.4	>99	A
<i>Mycobacterium sp.</i> BPCC 1186	42	4.8	42	>99	A
<i>Mycobacterium sp.</i> BPCC 1187	42	7.4	18.3	>99	A
<i>Arthrobacter petroleophagus</i> ATCC 21494	42	3.5	6.6	>99	A
<i>Brevibacterium paraffinolyticum</i> ATCC 21195	42	2.6	21.7	>99	A
<i>Ustilago maydis</i> BPFC 1198	18	6.7	45	>99	A
<i>Ustilago maydis</i> BPFC 6333	18	4.6	43	>99	A
<i>Aspergillus niger</i> BPFC 32	42	5.6	2.7	-	-
<i>Penicillium frequentans</i> BPFC 386	18	5	0	-	-
<i>Penicillium frequentans</i> BPFC 585	48	5.2	0	-	-
<i>Penicillium frequentans</i> BPFC 623	48	4.5	0	-	-
<i>Penicillium frequentans</i> BPFC 733	18	3.5	0	-	-

(E.e. means Enantiomeric excess)

5 **TABLE 6**

Microorganism	Reaction time (h)	Aqueous concentration (PPM)		E.e (%)	Enantiomer
		Compound of formula (Ie)	Compound of formula (IIe)		
<i>Mycobacterium sp.</i> BPCC 1179	42	1.6	3.3	>99	A
<i>Arthrobacter petroleophagus</i> ATCC 21494	42	3.2	0	-	-
<i>Brevibacterium paraffinolyticum</i> ATCC 21195	72	4.0	1.6	-	-
<i>Ustilago maydis</i> BPFC 1198	18	2.3	0	-	-
<i>Ustilago maydis</i> BPFC 6333	72	3.2	0	-	-
<i>Aspergillus niger</i> BPFC 32	72	3.7	9.2	-	-
<i>Penicillium frequentans</i> BPFC 386	72	3.1	0.5	-	-
<i>Penicillium frequentans</i> BPFC 585	48	3.2	3.2	-	-
<i>Penicillium frequentans</i> BPFC 623	48	2.9	1.5	83.4	B
<i>Penicillium frequentans</i> BPFC 733	18	3.2	0	-	-

The oxidation of the compound of formula (Id) produced in all cases the "A" enantiomer of the compound of formula (IId) in excellent enantiomeric excess but in low yield. The four strains of Penicillium frequentans, previously shown to oxidise the compound of formula (Ia), failed to oxidise the compound of formula  
5 (Id).

The oxidation of the compound of formula (Ie) produced fewer results. This compound proved to be particularly insoluble making the detection of product difficult. Whilst in a number of cases sulfoxide was produced, its concentration  
10 was too low to determine the enantiomeric excess. However two results were obtained with Mycobacterium sp. and Penicillium frequentans both affording sulfoxide of high enantiomeric excess but interestingly of opposite stereoselectivity.

### 15 EXAMPLE 3

The microorganisms listed in Table 9 below were screened for sulfoxidation activity against compounds of formula (Ib). They were grown under the same condition as in Examples 1 and 2.

20

Biotransformations were performed following the protocol of Example 1 except that the dry cell weight was increased to approximately 20gL<sup>-1</sup> and the reaction time was extended.

### 25 Detection of Products

The biooxidation of the compound of formula (Ib) was followed by reverse phase HPLC as in Example 1 except that the retention times were as follows:

**TABLE 7**

Compound of formula	Retention time (min)
Ib	8.1
IIb	4.2

The enantiomeric composition of the compound of formula (IIb) was investigated by the method of Example 1 except in the chiral HPLC the solvent composition,

5 flow rate and retention time were as follows:

**TABLE 8**

Solvent composition	Flow Rate (ml/min)	Retention times (min)
Hexane/ethanol (70:30%)	1.0	32.3 (Enantiomer A) 36.6 (Enantiomer B)

In Table 8 the first enantiomer eluted is referred to as enantiomer A and the  
10 second as enantiomer B.

The results are summarised in the following table:

**TABLE 9**

Microorganism	Reaction time (h)	Aqueous concentration (PPM)		E.e (%)	Enantiomer
		Compound of formula (Ib)	Compound of formula (IIb)		
<i>Mycobacterium</i> sp. BPCC 1178	72	8.6	3.4	8.2	B
<i>Brevibacterium paraffinolyticum</i> ATCC 21195	72	8.4	4.0	26.6	B
<i>Ustilago maydis</i> BPFC 6333	72	8.2	4.3	>99	A
<i>Aspergillus niger</i> BPFC 32	72	5.6	28.0	>99	A
<i>Penicillium frequentans</i> BPFC 386	72	8.4	4.5	-	-
<i>Penicillium frequentans</i> BPFC 585	48	6.5	11.4	-	-
<i>Penicillium frequentans</i> BPFC 623	48	7.7	6.5	-	-

(E.e. means enantiomeric excess)

- 5 The microorganisms listed in Table 9 were also screened under identical conditions for sulfoxidation of the compound of formula (Ic) but no product of formula (IIc) could be detected.

#### Deposits Of Microorganisms

- 10 The following microorganisms were deposited at the National Collections of Industrial and Marine Bacteria Ltd (NCIMB), 23 St. Machar Drive, Aberdeen, Scotland AB2 1RY on 25 November 1994:

- 15 1. Mycobacterium sp BPCC 1174  
Accession No. NCIMB 40695  
2. Mycobacterium sp BPCC 1178  
Accession No. NCIMB 40696  
3. Mycobacterium sp BPCC 1179  
Accession No. NCIMB 40697  
20 4. Mycobacterium sp BPCC 1186  
Accession No. NCIMB 40698  
5. Mycobacterium sp BPCC 1187  
Accession No. NCIMB 40699

- 25 The following microorganisms were deposited at the International Mycological Institute (IMI), Bakeham Lane, Englefield Green, Egham, Surrey TW20 9TY, England on 28 November 1994:

6. Penicillium frequentans BPFC 386  
Accession No. IMICC 364802  
7. Penicillium frequentans BPFC 585  
30 Accession No. IMICC 364801  
8. Penicillium frequentans BPFC 623

Accession No. IMICC 364800

9. Penicillium frequentans BPFC 733

Accession No. IMICC 364799

10. Rhizopus stolonifer BPFC 1581

5 Accession No. IMICC 364798

11. Ustilago maydis BPFC 1198

Accession No. IMICC 364797

12. Ustilago maydis BPFC 6333

Accession No. IMICC 364796

- 10 13. Asperigillus niger BPFC 32

Accession No. IMICC 364795

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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>20</u> , line <u>15</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution The National Collections of Industrial and Marine Bacteria Limited	
Address of depositary institution (including postal code and country) 23 St Machar Drive ABERDEEN AB2 1RY Scotland, United Kingdom	
Date of deposit November 25, 1994	Accession Number NCIMB 40695
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
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<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>20</u> , line <u>17</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b>	
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<b>Name of depositary institution</b> The National Collections of Industrial and Marine Bacteria Limited	
<b>Address of depositary institution (including postal code and country)</b> 23 St Machar Drive ABERDEEN AB2 1RY Scotland, United Kingdom	
<b>Date of deposit</b> November 25, 1994	<b>Accession Number</b> NCIMB 40696
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
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<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
<b>Name of depositary institution</b> The National Collections of Industrial and Marine Bacteria Limited	
<b>Address of depositary institution (including postal code and country)</b> 23 St Machar Drive ABERDEEN AB2 1RY Scotland, United Kingdom	
<b>Date of deposit</b> November 25, 1994	<b>Accession Number</b> NCIMB 40697
<b>C. ADDITIONAL INDICATIONS</b> <i>(leave blank if not applicable)</i> <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.	
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<b>B. IDENTIFICATION OF DEPOSIT</b>	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
<b>Name of depositary institution</b> The National Collections of Industrial and Marine Bacteria Limited	
<b>Address of depositary institution (including postal code and country)</b> 23 St Machar Drive ABERDEEN AB2 1RY Scotland, United Kingdom	
<b>Date of deposit</b> November 25, 1994	<b>Accession Number</b> NCIMB 40698
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
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<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution The National Collections of Industrial and Marine Bacteria Limited	
Address of depositary institution (including postal code and country) 23 St Machar Drive ABERDEEN AB2 1RY Scotland, United Kingdom	
Date of deposit November 25, 1994	Accession Number NCIMB 40699
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
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<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>20</u> , line <u>28</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364802
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
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<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364801
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364800
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<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>21</u> , line <u>3</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></div>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364799
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>	
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<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>21</u> , line <u>5</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364798
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
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<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="border: 1px solid black; padding: 5px;"> <input checked="" type="checkbox"/> For receiving Office use only  <input checked="" type="checkbox"/> This sheet was received with the international application         </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;">         Authorized officer  </div>	<div style="border: 1px solid black; padding: 5px;"> <input type="checkbox"/> For International Bureau use only  <input type="checkbox"/> This sheet was received by the International Bureau on:         </div> <div style="border: 1px solid black; padding: 5px; margin-top: 5px;">         Authorized officer       </div>
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A. The indications made below relate to the microorganism referred to in the description</b> on page <u>21</u> , line <u>7</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364797
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

<div style="text-align: right; font-size: small;">For receiving Office use only</div> <div style="display: flex; align-items: center; margin-bottom: 10px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border-top: 1px solid black; padding-top: 10px;">Authorized officer </div>	<div style="text-align: right; font-size: small;">For International Bureau use only</div> <div style="display: flex; align-items: center; margin-bottom: 10px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border-top: 1px solid black; padding-top: 10px;">Authorized officer</div>
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)


<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>21</u> , line <u>9</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364796
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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Authorized officer <i>Claire Hull</i>

<b>For International Bureau use only</b>
<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>21</u> , line <u>11</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution International Mycological Institute	
Address of depositary institution (including postal code and country) Bakeham Lane Egham Surrey TW20 9TY, England, UK	
Date of deposit November 28, 1994	Accession Number IMICC 364795
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism(s) be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), U.K. Rule 17(3), Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated state.	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div style="text-align: right; font-size: small;">For receiving Office use only</div> <div style="border: 1px solid black; padding: 2px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px;">Authorized officer </div>	<div style="text-align: right; font-size: small;">For International Bureau use only</div> <div style="border: 1px solid black; padding: 2px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border: 1px solid black; padding: 2px; margin-top: 5px;">Authorized officer</div>

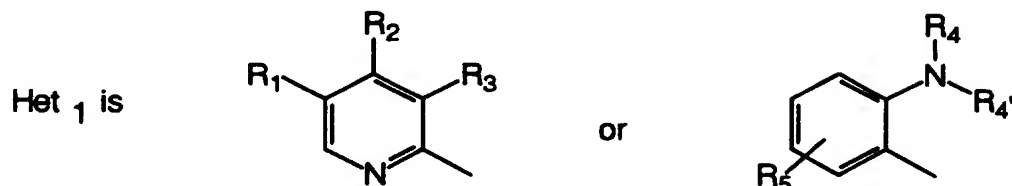
CLAIMS

1. A method of preparing a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

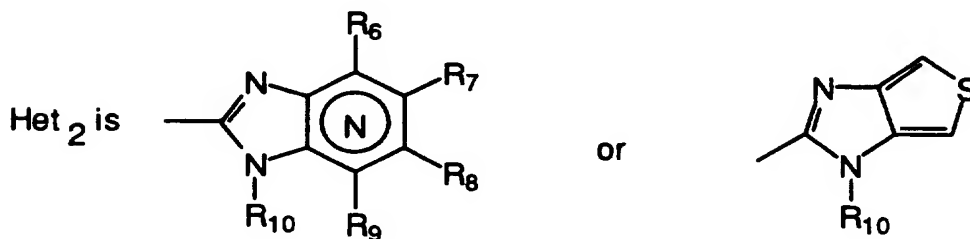


5

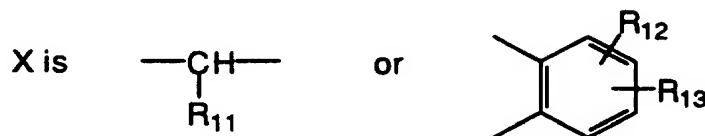
wherein:



and



10 and



wherein:

15 N in the benzimidazole moiety means that one of the carbon atoms substituted by R<sub>6</sub>-R<sub>9</sub>, optionally may be exchanged for an unsubstituted nitrogen atom;

$R_1$ ,  $R_2$  and  $R_3$  are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

- 5  $R_4$  and  $R_5$  are the same or different and selected from hydrogen, alkyl, aralkyl;

$R_6$  is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

- 10  $R_6 - R_9$  are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups  $R_6 - R_9$  may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

$R_{10}$  is hydrogen or alkoxycarbonyloxymethyl;

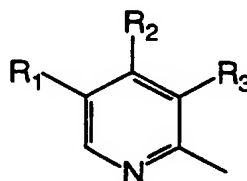
15

$R_{11}$  is hydrogen or forms an alkylene chain together with  $R_9$ ;

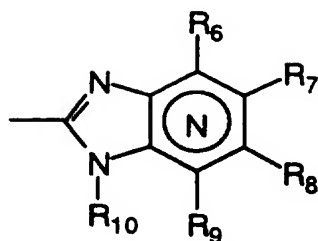
$R_{12}$  and  $R_{13}$  are the same or different and selected from hydrogen, halogen or alkyl; which method comprises stereoselective biooxidation of the pro-chiral sulfide  
20 counterpart compound.

2. A method according to claim 1 wherein:

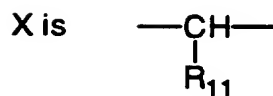
Het<sub>1</sub> is



25 and

Het<sub>2</sub> is

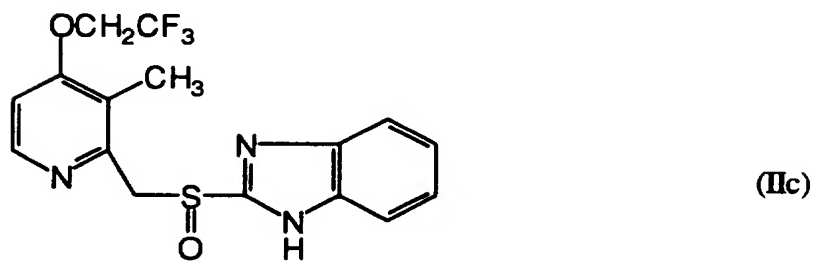
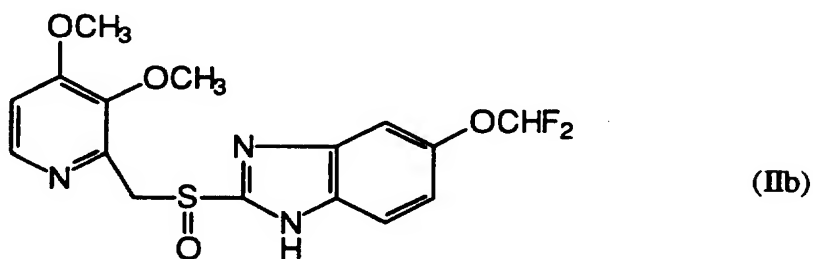
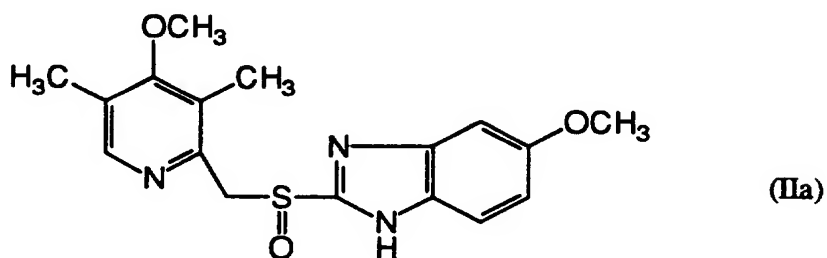
and



wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>6</sub>-R<sub>9</sub>, R<sub>10</sub> and R<sub>11</sub> are as defined in claim 1.

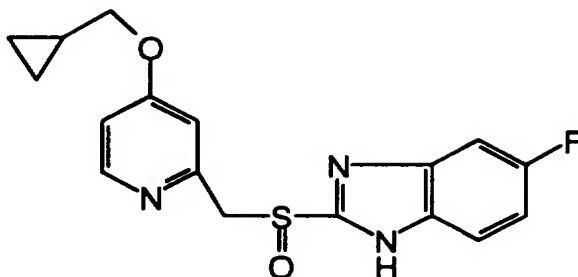
5

3. A method to claim 1 or 2 wherein the compound of formula (II) is a compound of formula:

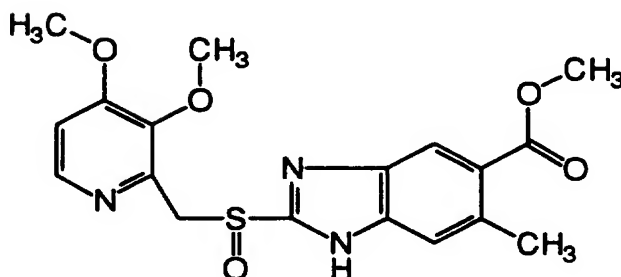


10

**SUBSTITUTE SHEET**



(IIId)



(IIc)

5 4. A method according to any one of the previous claims wherein a single enantiomer of the compound of formula (II) is prepared.

5. A method according to claim 3 wherein there is prepared a compound of formula (IIa) and the biooxidation is carried out with a microorganism which is

10

Penicillium frequentans

Brevibacterium paraffinolyticum or

Mycobacterium sp.

15 6. A method according to claim 3 wherein there is prepared a compound of formula (IIb) and the biooxidation is carried out with a microorganism which is:

Aspergillus niger or

Ustilago maydis.

20

**SUBSTITUTE SHEET**



7. A method according to claim 3 wherein there is prepared a compound of formula (II<sub>d</sub>) and the biooxidation is carried out with a microorganism which is

Mycobacterium sp.

5

Arthrobacter petroleophagus

Brevibacterium paraffinolyticum or

Ustilago maydis.

8. A method according to claim 3 wherein there is prepared a compound of  
10 formula (II<sub>e</sub>) and the biooxidation is carried out with a microorganism which is:

Mycobacterium sp.

Penicillium frequentans

15 9. A method according to claim 1 substantially as described in any one of the Examples.

10. A compound of formula II, as a single enantiomer or in enantiomerically enriched form, as defined in claim 1 prepared by the method claimed in any one  
20 of claims 1 to 9.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 95/01415

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 11/00, C07D 401/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA, MEDLINE, EMBASE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0005129 A1 (AB HÄSSLE), 31 October 1979 (31.10.79) --	1-4
A	Chem. Rev., Volume 88, 1988, H.L. Holland, "Chiral Sulfoxidation by Biotransformation of Organic Sulfides" page 473 - page 485 --	1-10
A	Drug Metabolism and Disposition, Volume 21, No 4, 1993, J.R. Cashman et al., "Chemical, Enzymatic and Human Enantioselective S-Oxygenation of Cimetidine" page 587 - page 597 --	1-10

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

19 March 1996

Date of mailing of the international search report

21 -03- 1996

Name and mailing address of the ISA/  
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Facsimile No. +46 8 666 02 86

Authorized officer

Gerd Strandell  
Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 95/01415

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Enzyme Microb. Technol., Volume 3, 1981, R.S. Philips, S.W. May, "Enzymatic sulphur oxygenation reactions" page 9 - page 18  -- -----	1-10

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

05/02/96

International application No.

PCT/SE 95/01415

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0005129	31/10/79	SE-T3- 0005129	
		AT-B- 374471	25/04/84
		AT-B- 374472	25/04/84
		AT-B- 374473	25/04/84
		AT-B- 375365	25/07/84
		AT-B- 389995	26/02/90
		AU-B,B- 529654	16/06/83
		AU-A- 4602779	18/10/79
		CA-A- 1127158	06/07/82
		CA-A- 1129417	10/08/82
		JP-C- 1312930	28/04/86
		JP-C- 1504537	13/07/89
		JP-A- 54141783	05/11/79
		JP-A- 58192880	10/11/83
		JP-B- 60034956	12/08/85
		JP-B- 63053191	21/10/88
		LU-A- 88307	04/05/94
		SE-A- 7804231	15/10/79
		SU-A,A- 895292	30/12/81
		US-A- 4255431	10/03/81
		US-A- 4337257	29/06/82
		US-A- 4508905	02/04/85

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